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(54) **Pharmaceutical compositions comprising selected lactobacillus strains.**

(57) Topical pharmaceutical compositions, suited for the use in gynecology and urology, comprise as active principles selected Lactobacillus strains isolated from vaginal or urologic habitat of asymptomatic patients.

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PHARMACEUTICAL COMPOSITIONS COMPRISING SELECTED LACTOBACILLUS STRAINS

The present invention concerns pharmaceutical compositions suited for the treatment of vaginal and urological diseases, containing selected strains of microorganisms of the "Lactobacillus" genus.

A further object of the invention is provided by said strains.

The use of lactic acid bacteria in gynecology has already been proposed (FR 6838 M and US 4592748).

Their use in urology has been disclosed, for instance, by:

a) Andrew W. BRUCE - Gregor REID: "Intravaginal instillation of lactobacilli for prevention of recurrent urinary tract infections";

b) Gregor REID - Andrew W. BRUCE - Mojtaba BEHESATI: "Effect of antibiotic treatment on receptivity of uroepithelial cells to uropathogens";

c) Gregor REID - Jacqueline A. McGROARTY - Rosanne ANGOTTI and Roger L. COOK: "Lactobacillus inhibitor production against "Escherichia coli" and coaggregation ability with uropathogens".

It has now been found that the use of particular strains of Lactobacillus, isolated from the vaginal habitat, taxonomically characterized and selected by means of "in vitro" activity tests, allow to obtain particularly favourable therapeutic results, thanks to:

(a) their high affinity towards the vaginal epithelium allowing their implant on the vaginal mucosa both in physiological and in pathological conditions, restoring therefore the optimal microflora and pH conditions;

(b) their direct action on the pathogenic microorganism, apparently not due to the production of diffusible inhibitory substances but to a coaggregation with the pathogenic microorganism.

Of course, the validity of the invention should not be considered to be connected with the verification of the above suggested mechanism of action.

The pharmaceutical compositions of the invention are particularly useful for the treatment of vaginal and urological affections of mycotic and bacterial origin.

The strains were deposited at the Collection National de Cultures de Microorganismes (CNCM) of Institut Pasteur - Paris (France) on July, 21, 1988;

The identity and deposit numbers of the strains of the invention is hereinbelow reported.

LACTOBACILLUS CASEI	I-785
LACTOBACILLUS GASSERI	I-786
LACTOBACILLUS FERMENTUM	I-789
LACTOBACILLUS CASEI SUBSP. PSEUDOPANTARUM	I-790
LACTOBACILLUS CRISPATUS	I-787
LACTOBACILLUS FERMENTUM	I-788

Some information relevant to the characterisation of the strain of the invention are hereinbelow reported.

Lactobacillus Casei : I-785

Guanine + cytosine content (G + C %): 39.7%

Fermented sugars according to API CHL 5041 test:

L-arabinose

ribose

galactose

glucose

fructose

N-acetylglucosamine

maltose

melibiose

sucrose

mellicitose

raffinose

gluconate
5-oxo-gluconate
Plasmids: 1 plasmid 30M daltons
Surface protein (S-layer): Molecular weight 56-66.000 daltons.

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Lactobacillus Gasserl : I-786

G + C %: 34.5
Fermented sugars according to API CHL 5041 test:

10 galactose

glucose

fructose

mannose

N-acetylglucosamine

15 amygdaline

arbutine

salicine

cellobiose

maltose

20 sucrose

trehalose

gentiiose

D tagatose

Plasmids: 1 32M daltons

25 Surface protein: two bands with Molecular weight = 50.000

Hybridization with Lactobacillus Gasserl DSM 20043:78%

Lactobacillus Fermentum : I-789

30 G + C %: 51.2

Fermented sugar according to HPI CHL 5041 test:

L-arabinose

ribose

galactose

35 glucose

fructose

mannose

maltose

lactose

40 melibiose

sucrose

raffinose

gluconate

5-oxo-gluconate

45 Hybridization with Lactobacillus Fermentum ATCC 14932:100%

Lactobacillus Casei supsp.Pseudopantarum : I-790

G + C%: 46

50 Hybridization with ATCC 2558:92%

Lactobacillus Crispatus: I-787

G + C %: 38

55 Hybridization with ATCC 33820:80%

Lactobacillus Fermentum (LF4): I-788

G + C%: 52.2

Hybridization with ATCC 14931

The compositions of the invention may comprise one or more strains selected in the above defined group.

Particularly favourable therapeutic results were obtained with the following mixtures:

MIXTURE A 1	
<u>Lactobacillus Casei</u>	I-785
<u>Lactobacillus Gasseri</u>	I-786
<u>Lactobacillus Fermentum</u>	I-789
MIXTURE A 2	
<u>Lactobacillus Casei subsp. Pseudopiantarum</u>	I-790
<u>Lactobacillus Crispatus</u>	I-787
<u>Lactobacillus Fermentum</u>	I-788

Of course, other mixtures or single strains may be advantageously used in the compositions, comprised within the invention's scope.

A particularly preferred strain is *Lactobacillus fermentum* I-789, which proved to be endowed with a direct inhibitory action on some species of pathogenic fungi, particularly on *Candida* strains, as it has been shown by means of *in vitro* tests on agar plates whereupon *Candida* strain was contacted with *Lactobacillus Fermentum* I-789. An inhibitory halo of *Candida* was noticed and a test carried out using the sterile supernatant of the I-789 culture instead of the whole cells shows that said inhibitory action is not due to inhibitory substances released in the culture medium.

For the practical use, the microorganisms of the invention are formulated in suitable administration forms such as ovules, creams, vaginal capsules, solutions for lavages, sachets and the like. Unit doses may comprise from 10^3 to 10^{10} cells of each single strain, the preferred dosage being about 10^6 cells per unit dose.

The bacterial cultures are preferably in lyophilized forms and may be prepared according to conventional methods.

For the preparation of aqueous formulations for lavages and irrigations small bottles are used, provided with reservoirs containing the lyophilized microorganisms, to be dissolved before use in a suitable liquid carrier contained in the bottles.

The compositions of the invention are practically devoid of any toxicity and show no systemic absorptions so as to allow the administration also to pregnant patients and in cases of intolerance to antimycotic or antibacterial drugs.

The compositions of the invention are therefore particularly useful, *inter alia*, for the prophylaxis of fungal infections due to antibiotic treatments and corresponding complications (formation of resistant strains, relapses etc.). The clinical experience up to now acquired shows as particularly convenient and effective a treatment schedule comprising the administration of the composition of the invention in form of capsules, creams or ovules to be administered before sleeping followed by a lavage on the subsequent morning.

The following non limitative examples further illustrate the compositions of the invention.

EXAMPLE 1	
OVULES	1 x 10^6 cells of each strain
Active principle	
Mixture A 1	
Excipient	
Semi-synthetic glycerides	2800 mg

EXAMPLE 2

VAGINAL CREAM (30 g tube)	
Active principle	
Lactobacillus fermentum I-789	1 x 10 ⁶ cells of each strain
Excipient	
Hydrogenated lanoline	5 g
Vaseline oil	5 g
Dimethylpolysiloxane	10 g
SiO ₂ (Aerosil 200 ^R)	15 g
Dodecylglycol polyethylenglycol	1,5 g
1000 copolymer	6 g

EXAMPLE 3

VAGINAL CAPSULES	
Active principle	
Mixture A 1	1 x 10 ⁶ cells of each strain
Excipient	
Triglycerides with C ₈ -C ₁₂ fatty acids (Migliol ^R)	1500 mg
Glycerine	2500 mg
Titanium dioxide	10 mg

EXAMPLE 4

BOTTLES WITH RESERVOIR	
Each bottle contains:	
a) RESERVOIR	
Lyophilized mixture A 1	1 x 10 ⁶ cells of each strain
b) BOTTLE (10 ml)	
Glycerine	4,0 g
Water q.s. to	10 ml

EXAMPLE 5	
SOLUTION FOR LAVAGES	
Each bottle with reservoir contains:	
a) RESERVOIR	
Lyophilized mixture A 2	1 x 10 ³ cells of each strain
b) BOTTLE (150 ml)	
Glycerine	60 g
Water q.s. to	150 ml

Claims

1. A Lactobacillus strain deposited at C.N.C.M. of Institut Pasteur on 21.7.1988 having the following identity and deposit number.
2. Pharmaceutical compositions containing at least a Lactobacillus strain of claim 1.
3. Pharmaceutical compositions according to claim 2 containing a mixture of Lactobacillus strains of claim 1.
4. Pharmaceutical compositions according to claims 1 or 2 containing from 10³ to 10¹⁰ cells of each strain per unit dose.
5. Pharmaceutical compositions according to claim 4 containing about 10⁶ cells of each strain per unit base.
6. A pharmaceutical composition according to any one of the preceding claims containing Lactobacillus fermentum I 789.
7. Pharmaceutical compositions according to any one of claims 1-5, containing a mixture of:

- Lactobacillus Casei	I-785
- Lactobacillus Gasseri	I-786
- Lactobacillus Fermentum	I-789

8. Pharmaceutical composition according to any one of claims 1-5, containing a mixture of:

- Lactobacillus Casei Pseudopiantarum	I-790
- Lactobacillus Crispatus	I-787
- Lactobacillus Fermentum	I-788

9. Pharmaceutical composition according to any one of the preceding claims in form of creams, ovules, vaginal capsules, solutions for lavages.